

ANTI-THYROID HORMONE (T4) RECOMBINANT ANTIBODY OR ANTIGEN BINDING FRAGMENT

FIELD OF THE INVENTION

[0001] The present invention relates to the fields of antibodies, antigen binding fragments and immunodiagnostics. Specifically, the invention relates to a recombinant antibody or antigen binding fragment for binding thyroid hormone T4 (thyroxine) and halogenated bisphenol A. Also, the present invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes the recombinant antibody or antigen binding fragment of the present invention, as well as an expression vector and host cell comprising the nucleic acid molecule of the present invention. Still, the present invention relates to a method of producing a recombinant antibody or antigen binding fragment for binding T4 thyroid hormone and halogenated bisphenol A, a test kit and an immunoassay comprising the recombinant antibody or antigen binding fragment of the present invention, and a method for determining T4 thyroid hormone and/or halogenated bisphenol A levels in a sample of a subject. Still further, the present invention relates to a method of treating a sample, e.g. an immunoaffinity-based sample preparation method for enrichment of the halogenated bisphenol A from a sample.

BACKGROUND

[0002] Thyroid hormones have an important role in differentiation, growth and metabolism. The thyroid hormones, triiodothyronine (T3) and thyroxine (T4) are produced by the thyroid gland. The precursors for T4 are tyrosine amino acids of the thyroglobulin expressed in thyroid gland. Tyrosine amino acids are combined with iodine, two iodinated tyrosines are conjugated and subsequently cleaved from the thyroglobulin. T4 is further converted to T3 by deiodinase enzymes within the target cells. The difference between T4- and T3-hormones is only one iodine atom.

[0003] T4 is the dominant thyroid hormone in serum with concentration ca 40 fold higher than T3 (90 nM and 2 nM, respectively). Only 0.03% of the total T4 in serum (10-25 pM) and 0.3% of T3 (4.0-8.0 pM) is in an unbound form which enters the cells and has the biological activity (Yen, P. M. *Physiological Reviews* (2001) 81:1097-1142).

[0004] At the moment, the commonly used test for analyzing T4 from serum is a radioimmunoassay (RIA). The determination of the free T4 is an important tool for screening of the potential thyroid dysfunction. When unusual values are obtained then more thorough diagnostics to clarify the cause of thyroid gland dysfunction is performed (free and bound T4, T3, TSH and autoantibodies). In some disorders increasing amounts of T3 are produced while T4 levels are normal. Therefore, it is important to have multiplexed diagnostics to have a comprehensive picture of thyroid gland function.

[0005] T4 has one iodine more than T3. This minor difference between the most important thyroid hormones makes the specific antibody discovery for either T3 or T4 very challenging. In addition, both thyroid hormones T3 and T4 are relatively small and hydrophobic.

[0006] Several commercial antibody products, either polyclonal or monoclonal, are available for thyroid hormones. Also, several patent applications relate to thyroid hormone anti-

bodies. E.g. JP2010178649 (A) describes an anti-thyroxine antibody comprising a specific heavy chain variable region (V_H) and a specific light chain variable region (V_L). Islam et al (2011) describes an antibody for T4 which in recombinant Fab-format was selected using a phage display technology and used for the one-step open immunoassay (Islam K N et al. *Analytical Chemistry* (2011) 83: 1008-1014).

[0007] Still, antibodies or antigen binding fragments having especially high affinity for T4 are needed for determination of thyroid hormone T4 in a biological sample.

SUMMARY OF THE INVENTION

[0008] The present invention concerns a novel reagent for rapid and reliable detection of T4 thyroid hormone. Also, the present invention concerns a novel reagent for rapid and reliable detection of halogenated bisphenol A.

[0009] The present invention reveals surprising binding properties of an antibody or antigen binding fragment capable of binding thyroid hormones and halogenated bisphenol A. Indeed, the present invention relates to a recombinant antibody or antigen binding fragment capable of binding thyroid hormone T4 with high affinity and capable of halogen dependent binding to halogenated bisphenol A.

[0010] One surprising advantage of the present invention includes that observed levels of halogenated bisphenol A in humans do not disturb the immunoassay when high affinity anti-T4 antibody is used. However, the cross reactivity of the T4 thyroid hormone binding antibody or antigen binding fragment of the present invention for the halogenated bisphenol A makes it possible to use the same antibody for the measurement and/or sample preparation of halogenated bisphenol A from e.g. biological, environmental or food samples. Cross-reactivity also provides efficient tools for evaluating the endocrine disruptor potential of organic molecules having effect on the thyroid hormone system.

[0011] The present invention relates to a recombinant antibody or antigen binding fragment (e.g. isolated antibody or antigen binding fragment) for binding T4 thyroid hormone and halogenated bisphenol A.

[0012] Also, the present invention relates to an isolated (recombinant) nucleic acid molecule comprising a nucleotide sequence that encodes the recombinant antibody or antigen binding fragment of the present invention.

[0013] Also, the present invention relates to an expression vector comprising the nucleic acid molecule of the present invention.

[0014] Still, the present invention relates to a host cell comprising the nucleic acid molecule or the expression vector of the present invention.

[0015] Furthermore, the present invention relates to a method of producing a recombinant antibody or antigen binding fragment for binding T4 thyroid hormone and halogenated bisphenol A (e.g. triiodobisphenol or tetrahalobisphenol A) of the present invention, wherein the method comprises introducing an expression vector of the present invention into a host cell and growing the cell under conditions permitting production of the antibody or antigen binding fragment.

[0016] Furthermore, the present invention relates to a test kit comprising the recombinant antibody or antigen binding fragment of the present invention. Also, use of said test kit for detecting T4 thyroid hormone and/or halogenated bisphenol A in a sample is within the scope of the present invention. Furthermore, use of a kit comprising the recom-